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## Neuropsychological function in manganese alloy plant workers

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**Abstract Objectives:** The objective was to investigate potential nervous system effects of manganese (Mn) exposure in workers employed in manganese-alloy-producing plants. **Methods:** One hundred male Mn alloy plant workers were compared with 100 age-matched referents. The subjects were examined with a comprehensive neuropsychological test battery. Exposure was assessed by measurement of Mn concentrations in the workroom air, blood and urine. **Results:** The geometric mean (GM) concentration of inhalable Mn in workroom air was 301  $\mu\text{g}/\text{m}^3$ . The GM concentration of Mn in whole blood (181 nmol/l vs 160 nmol/l) ( $P=0.002$ ) and urine (0.9 nmol/mmol creatinine vs 0.4 nmol/mmol creatinine) ( $P<0.001$ ) was higher among the exposed subjects than among the referents. The Mn-exposed subjects had increased postural tremor while conducting a visually guided tremor test (static steadiness test) compared with the referents (mean number of contacts 94 vs 59 ( $P=0.001$ ); duration of contacts (in seconds) 5.1 vs 3.5 ( $P=0.003$ )). The tremor had larger frequency dispersion, indicating that the tremor included a wider variety of frequencies, among the exposed subjects than among the referents, assessed by the "TREMOR" test system. Smoking habits (self-reported) influenced the tremor parameters significantly, the Mn-exposed smokers having more tremor than the non-smoking Mn-exposed subjects. No differences between the groups were found in tests for cognitive functions, reaction time or in symptom reporting. **Conclusions:** The Mn-exposed subjects had increased hand tremor compared with their referents. The tremor was related to exposure parameters. Smoking habits (self-reported) influenced the tremor parameters.

**Keywords** Tremor · Manganese exposure · Smoking · Neuropsychological · Neurobehavioural

### Introduction

Manganese (Mn) is an essential trace element but also a neurotoxicant. High exposure may lead to manganism, which is described as starting with neurological and psychiatric signs and symptoms. The end stage, first described by Couper [7], is severe neurological impairment affecting mainly the motor system [40, 45]. Severe disturbances from manganism can be observed clinically. This is not the case at lower exposure, when the anticipated effects are smaller. Neuropsychological tests are sensitive methods for detecting early functional impairments of the nervous system, and during the past 20 years, several neuropsychological/neurobehavioural studies [30] of Mn-exposed workers have been published [5, 6, 8, 11, 19, 27, 28, 29, 35, 36, 37, 41, 47, 48, 49, 50].

Depending on which neurobehavioural tests have been used, different central nervous system (CNS) effects are suggested as early signs of Mn toxicity. Some studies have reported group differences in total reaction time (RT) [29, 48, 49], whereas others have been non-positive with regard to total RT [35, 37, 50]. Some studies report effects on other RT parameters [41, 47]. Several studies report poorer performance among Mn-exposed workers on motor tests that can be classified as tests for eye-hand coordination, motor speed or manual dexterity [4, 6, 29, 35, 37, 41, 47, 48, 50]. With one exception [19], all studies where hole tremometers have been used report increased hand tremor among the exposed subjects compared with the referents [27, 41, 47] and/or association between exposure and effect on tremor parameters [8, 47, 48]. Studies using computer-assisted tremor tests have also reported effects on tremor parameters from Mn-exposure [27, 37]. Inferior performance has been reported among Mn-exposed subjects on tests for cognitive function, including memory tests [6, 35, 37, 41, 47].

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A moderate increase [6, 29, 37, 47, 50], a substantial increase [41], or no increase [19, 48] in neuropsychiatric symptoms have been reported.

The neurotoxic mechanisms of Mn have not been fully elucidated [1]. In the brain, Mn is accumulated mainly in the striatum, the subthalamic nuclei and the globus pallidus [2]. Manganese is cleared from the brain, and increased levels of Mn are not necessarily found in affected brain tissues [43, 54]. In Mn-intoxicated humans, damage to nerve cells is found mainly in the globus pallidus, and often in the striatum as well [43, 54]. The clinical picture of manganism resembles that found in disorders described as extrapyramidal, in particular Parkinson's disease (PD). However, neurodegeneration in PD occurs primarily in the substantia nigra pars compacta [43], in contrast to manganism, where the nigrostriatal pathway is usually preserved [1, 43].

The aim of the present study was to investigate potential nervous system effects of occupational manganese exposure in workers employed in manganese-alloy-producing plants.

## Subjects and methods

### Subjects

The three plants studied employ approximately 700 workers. Two plants produce ferro-manganese (FeMn) and silico-manganese (SiMn) alloys, while the third produces only SiMn. In FeMn production, the charge consists of manganese lump ore, iron ore, coal and coke as reductants and limestone as a flux. The slag formed during FeMn production is reused for producing SiMn, where it is smelted with quartzite using coke and coal as the reducing agents and dolomite as a flux. The alloys are produced by the heating of the raw materials in furnaces at temperatures from 500 to 1,500°C. Electricity is used to provide the required heat [52].

The study had a cross-sectional, matched-pair design and was restricted to include men only, as few women were employed in the plants. Subjects with known substance or alcohol abuse as documented in personnel files by the occupational health services (OHS) were not invited. Seventeen Mn-exposed subjects were not considered for inclusion due to this restriction. Further, the study was restricted to exposed subjects currently employed in the production or maintenance departments, and at least one year of employment was required. Exposed subjects and referents with occupational exposure to organic solvents (>5 years) or to lead or mercury (>1 year), were not invited. Subjects with current or previous serious neurological disease not known to be related to Mn exposure, as documented in personnel files by the OHS, were also not invited.

From an alphabetical list of the 547 identified eligible employees, every fourth worker was invited until the target of 100 subjects was met. In order to get 100 exposed subjects to attend the examinations, we invited 110. The study population of Mn-exposed subjects was made up proportionally to the workforce in each plant; 49% were recruited from the largest plant where 48.4 of the eligible subjects worked, 26% and 25% of the subjects were recruited from the plants employing 28.3% and 23.2% of the eligible subjects, respectively. Sixty-eight percent of the included exposed subjects were production workers, and 32% were maintenance workers.

The referents were recruited from two plants with working conditions similar to the Mn alloy plants; one plant produced silicon metal and microsilica, the other, titanium dioxide slag and pig iron. The referents were selected on the basis of pair matching

1:1 for age. After inclusion of an exposed subject, the referent with the closest birth date was invited to participate. If this subject declined participation, the person second closest in birth date was invited. In order to get the 100 required referents, we invited 110 subjects.

Occupational history and background characteristics were reported by the subjects in a brief (15-min) interview conducted by the same interviewer throughout the study. Current alcohol consumption was calculated, based on self-reported alcohol consumption [23]. All subjects volunteered to participate in the study, and their written informed consent was obtained. The study was approved by the Regional Ethical Committee for Medical Research.

### Exposure assessment

The ongoing exposure to airborne Mn was characterised by personal, full-shift sampling, ideally on 3 days for each individual, closely before and/or after the day of the neuropsychological examination. In all, 265 personal samples (from 100 exposed subjects) of the inhalable aerosol fraction (IOM sampler, SKC, Pennsylvania, USA), and 167 respirable samples (from 59 exposed subjects) (Casella cyclones, Casella, London, England) were collected. Fifty-eight samples of the inhalable aerosol fraction were collected randomly among production and maintenance workers in the reference plants as well. Further details have been published [15].

A four-step chemical fractionation procedure was applied for speciation of Mn in a selection of the samples [51]. The components that dissolved in 0.01 mol/l ammonium acetate, in 25% acetic acid, and in 0.5% hydroxylamine hydrochloride/25% acetic acid were regarded as "soluble". The components measured after digestion in aqua regia/hydrofluoric acid were regarded as "insoluble". The individual dose estimates of current exposure to Mn were calculated as the mean concentration of the Mn determined on the individual filters.

### Measurements of trace elements in urine and whole blood

The subjects were instructed to bring a sample from home of first-voided morning urine from the day of the neuropsychological examination in 25-ml NUNC containers (Nalge Nunc International, Denmark). Heparinised whole blood was collected between 0800 and 0900 with 10-ml Venoject (Terumo Corp., Belgium) tubes. All samples were stored at -20°C until required for analysis.

*Manganese in urine (U-Mn)* was measured after dilution to 1:1 with 0.5% nitric acid by electrothermal atomic absorption spectrophotometry (EAAS) using a Perkin-Elmer SIMAA 6000 system calibrated with urine-matched standard solutions.

*Manganese in whole blood (B-Mn) and lead in whole blood (B-Pb)*. For the measurement of B-Mn and B-Pb, 2.5 ml of ultrapure 65% nitric acid was added to 2 ml of whole blood in a polypropylene digestion tube. The tube was heated to 95°C for 1 h, and after being cooled the sample was diluted to volume (13.7 ml). The solution was analysed simultaneously for Mn and Pb by EAAS.

*Mercury in urine (U-Hg) and in whole blood (B-Hg)* was measured by direct cold-vapour atomic absorption spectrometry. The blood samples were digested with nitric acid-hydrogen peroxide in Teflon autoclaves.

The accuracy and reproducibility were assessed with Seronorm human urine (batch 4043125) and whole blood (batches 404107 and 404108) reference materials (Sero, Asker, Norway). U-Mn and B-Mn were measured in these materials with a day-to-day variation of 6.5%, 3.5% (in 404107) and 5.0% (in 404108), respectively. The day-to-day variation of U-Hg and B-Hg were typically 1.5% and 5%, respectively. The reference materials results for B-Pb showed typically day-to-day variations of  $\leq 7\%$  and accuracies within  $\pm 10\%$ . As described elsewhere [15], all results agreed well with the recommended values.

## Neuropsychological methods

The neuropsychological examination was undertaken on the premises of the OHS, located close to the worksites. The subjects were requested in advance to wear proper visual correction, if needed. The same certified specialist in clinical neuropsychology examined all subjects. The plants were located in different geographical regions, and it was not feasible for a blind study to be conducted. The hour on the clock when the subjects underwent the examination was recorded. For shift workers, the time since the last night shift was recorded. Participants who were examined during their leisure time were paid. Raw scores were used in the statistical analyses.

### Symptoms

Subjective symptoms were recorded by means of two self-administered neuropsychiatric questionnaires. The Q16 [38] contains 16 questions, and the measure was the total number of symptoms. The "Helsinki Symptom Questionnaire" [24] contains 31 items with three options, given the loadings 1 (hardly ever), 2 (sometimes), and 4 (often) [50]. The answers were combined into six symptom scales [24].

### Cognitive functions

*General intellectual ability Information* [Wechsler's adult intelligence scale (WAIS)] [17] requires the subject to answer a number of questions requiring general knowledge. We included the test, to adjust for pre-exposure general intellectual ability.

*Visuo-motor speed and attention Digit Symbol* (WAIS) [17] requires the subject to recode symbols to digits in the course of 90 s. The measure was the number of correct symbols recoded.

*The Trail Making test* (TMT) [34, 44] requires the subject to draw a "trail" from one digit to another with a pencil (TMT A) or alternate between letters and digits (TMT B). The measure was time to complete the test (in seconds).

*Stroop test* [34]. This version has three cards, each with 48 items, which are to be responded to as quickly as possible. The first and second cards show coloured dots, which are to be named, and colour words, which are to be read, respectively. The third card shows 48 colour words, written in incongruent colours. The subject is asked to name the colour of each word, ignoring the word information. The measure was time to complete the test (in seconds).

*Immediate memory/attention span Digit Span* (WAIS) [17] requires the subject to repeat as many digits as possible after an oral presentation, either in the same order (Digits Forwards), or in reverse order (Digits Backwards) as presented. The measure was maximum number of digits repeated.

*Benton Visual Retention test* form C, administration A [34] consists of ten cards with increasingly complex visual designs. The subject is required to reproduce the design from memory on blank sheets of paper immediately after a 10-s presentation. The number of correct reproductions and errors were recorded.

### Motor tests

*Hand steadiness/tremor Kløve-Matthews Static Steadiness test* (Kløve-Matthews Motor Steadiness battery) [39] is a stylus-and-hole apparatus. The subject is required, without support, to hold the stylus in successively smaller holes, keeping it in each of nine holes for 15 s without it's touching the sides. Only the dominant hand was tested. The measures were cumulative number and duration of contacts (in seconds) between stylus and base plate.

"TREMOR" version 7.0 [Danish Product Development (DPD)] [10] requires the subject to hold the arm bent at an angle of 90° without any support and to hold a light tremor pen, containing a biaxial micro-accelerometer, like an ordinary pen. The default test

time of 10.2 s, 2 s for stabilisation and 8.2 s for data recording, was used. Three measures were applied: (1) tremor intensity (I), root mean square of the recorded accelerations (in metres per second squared); (2) centre frequency (F50); 50% of the power lies above and 50% below this frequency (in hertz); (3) dispersion of power (SF50), 68% of the power dissipated within  $F50 \pm SF50$  (in hertz). A fourth [harmonic index (HI)] and a fifth [tremor index (TI)] measure were not used as they are based on the three previous measures [5]. The HI has also been suggested to be unreliable in test-retest experiments [13].

*Tests of motor speed, grip strength, coordination and reaction time Finger Tapping test* [44] requires the subject to press a tapping key as fast as possible with the index finger. A manual version (Lafayette Instrument Company) was used. Only the dominant hand was tested. The measure was the median number of presses based on four trials, each lasting 10 s.

*Foot Tapping test* [39] requires the subject, while standing, to press a lever with the foot as quickly as possible for 10 s. The measure was the average number of presses for the dominant foot based on two trials.

*Dynamometer* (Lafayette Psychological Instruments, model 78010) [44] requires the subject to hold the upper part of the dynamometer in the palm of the hand and to squeeze the stirrup with the fingers as hard as possible. The measure was the amount of pressure in kilogrammes.

*Grooved Pegboard test* [39] consists of a small board containing a 5x5 set of slotted holes angled in different directions and 25 pegs with a ridge along one side. The subject is required to rotate the peg into position for correct insertion as quickly as possible. The score was time to completion in seconds for each hand.

*CATSYS System 7.0 (DPD)* [9]. Following an accelerating rhythm from 1.6 to 7.5 Hz, the subject is required to hit the surface of a touch-sensitive recording board lightly with the open hand, alternating between the back and the palmar side (supination/pronation of the hand). The maximum frequency at which the subject could follow the sound was recorded. Only the dominant hand was tested.

*Luria-NebraskaThumb/Finger Sequential Touch* [20] requires the subject to touch the thumb with the fingers of the same hand as fast as possible for 10 s. The measure was number of sequences for each hand.

*Simple Reaction Time test* (SRT) (NES2) [3] requires the subject to press a button as quickly as possible when a square appears on a computer screen. The inter-trial interval was varied at random. Ninety reaction times were recorded and the first ten were discarded. The measures were the mean RT and SD in milliseconds.

*Hand Eye Coordination test* (HECT) (NES2) [3] requires the subject to trace a cursor over a sinus-wave pattern on the computer screen. The subject controls the vertical motion with the joystick. The measure was the average distance of the cursor from the set line.

Version 4.51 of NES2 was used on a 80386 PC. All computerised tests (TREMOR, CATSYS, SRT, HECT), were run from the C:\ drive on the PC. The stimuli were presented on a 14-in. screen.

### Statistics

We log-transformed continuous variables when not normally distributed (defined as skewness exceeding 2.0), to achieve normalisation. With the exception of some exposure measures and some tremor parameters (Table 8), the arithmetic mean (AM) is presented in the tables, even when the log-transformed values were used in the statistical analyses for calculating *P* values. Student's *t*-test was used for group comparisons for continuous variables and the chi-square test for categorical variables.

We used multiple linear regression analysis (backwards procedure) to assess the influence of potential confounders on the dependent variables in all subjects (*n* = 200). Age, verbal ability

(raw scores on the test "information"), alcohol consumption (litres of pure alcohol per year), tobacco smoking (grammes per week), medication (yes/no), history of brain concussion (yes/no), shift work (yes/no), and whether exposed to Mn (yes/no) were included as independent variables.

The dependent variables where exposure status (yes/no) contributed significantly or nearly significantly ( $P < 0.10$ ) to the statistical model after assessment of potential confounding were analysed further. Only the exposed subjects ( $n = 100$ ) were included in the multiple linear regression analyses (backwards procedure) to assess the statistical relations between the neuropsychological test results, potential confounders and the exposure-related variables. We entered the exposure variables B-Mn, U-Mn, current exposure to "inhalable Mn" (IOM), current exposure to "inhalable soluble Mn" (IOM<sub>soluble</sub>) and years of exposure to Mn separately into the models to avoid collinearity.

Further, the exposed subjects were stratified into three equally large groups according to the above exposure parameters. Three groups were regarded as acceptable, based on calculations of statistical power. Based on the pair-wise matching by age of the exposed subjects and the referents, they could be compared with their age-matched referents. We used ANCOVA, including age and amount of smoking as covariates, to assess differences between the exposed and their age-matched referents ( $n = 200$ ) in the "low", "medium" and "high" exposure groups.

The level of significance was set at 0.05 (two-tailed). The statistical analyses were carried out with SPSS 6.1 for Windows.

## Results

Both groups had comparable background characteristics (Table 1). The concentrations of B-Mn, U-Mn and B-Pb were higher in the Mn-exposed workers than in the referents (Table 2). The exposure to inhalable Mn in the reference plants was AM  $6 \mu\text{g}/\text{m}^3$  (GM  $4 \mu\text{g}/\text{m}^3$ , 95% CI 3-5;  $n = 58$ ) (not tabulated). As the respirable aerosol fraction was not sampled for all participants, only a limited number of exposed subjects could be assigned a respirable "manganese exposure dose".

The mean number of symptoms on the Q16 and on the six symptom subscales on the Helsinki Symptom Questionnaire were comparable in the two groups (Table 3). One symptom was reported more often on the Q16 among the exposed subjects than among the referents: "Do you often have to make notes about what you must remember?" ( $P < 0.001$ ). On the Helsinki Questionnaire, more referents reported "sometimes or often" feeling weakness in their legs ( $P = 0.04$ ). Shift workers

reported more symptoms on the Q16 (3.0 vs 2.1,  $P = 0.02$ ) and had a higher symptom score on "memory and concentration difficulties" (1.8 vs 1.6,  $P = 0.048$ ) than non-shift workers.

Cognitive speed, attention or short-term memory was comparable in the two groups (Table 4), as was also the number of errors on the TMT and the number of errors and near-misses on the Stroop test (not tabulated). The WAIS subtest information, included to adjust for pre-exposure general intellectual ability, discriminated nearly significantly ( $P = 0.06$ ) between the groups.

The Mn-exposed subjects had more tremor than the referents on the Static Steadiness test (Table 5). Both groups had tremor of similar frequency, approximately 7.5 Hz. For both hands, the frequency dispersion was larger among the exposed subjects. The difference in frequency dispersion between the dominant and non-dominant hand was larger in the referents than in the exposed subjects. The test for hand supination/pronation, maximum frequency, discriminated significantly between the groups (Table 6).

Both measures of the Static Steadiness test were associated with exposure status ( $P < 0.001$ ), increasing age ( $P < 0.001$ ) and number of cigarettes/week ( $P < 0.01$ ) after we had taken into account the potential confounders in the regression analysis of all subjects ( $n = 200$ ). Exposure status was also associated with larger frequency dispersion on "TREMOR" after inclusion of the covariates for the dominant hand ( $P < 0.001$ ), but not for the non-dominant hand ( $P = 0.11$ ). Cigarette smoking was associated with smaller frequency dispersion (not tabulated).

Test results associated with exposure status ( $P < 0.10$ ) in the regression analyses including all subjects ( $n = 200$ ) were analysed further so that we could study dose-effect in the Mn-exposed subjects ( $n = 100$ ). Exposure duration and weekly consumption of cigarettes were associated with more tremor on both measures of the Static Steadiness test. The regression equations (1) number of contacts (log) =  $1.5^{P < 0.001} + 0.01^{P < 0.05}$  years exposed +  $0.002^{P < 0.001}$  cigarettes/week and (2) duration of contacts (s) =  $1.1^{P > 0.05} + 0.15^{P < 0.01}$  years exposed +  $0.02^{P < 0.01}$  cigarettes/week, were calculated. The  $R^2$  of both models were 0.16. Figure 1 shows the test

**Table 1** Background characteristics for the Mn-exposed subjects and the referents

Characteristic	Exposed ( $n = 100$ )			Referents ( $n = 100$ )			P
	Mean	SD	Minimum-maximum	Mean	SD	Minimum-maximum	
Age (years)	44.2	9.0	27.6-61.9	44.2	9.0	27.7-61.4	0.98
Years of education	10.9	2.1	7.0-17.0	11.4	2.2	7.0-17.5	0.09
Prevalence of shift workers (%)	42	-	-	50	-	-	0.26
Self-reported alcohol consumption (l/year)	3.9	3.8	0-24.9	3.3	3.4	0-15.9	0.27
Prevalence of smokers (%)	50	-	-	45	-	-	0.48
Tobacco consumption (g/week)	41	56	0-225	41	57	0-225	0.99
Prevalence of self-reported use of medication (%)	22	-	-	25	-	-	0.62
Prevalence of self-reported earlier brain concussions (%)	25	-	-	24	-	-	0.87
Prevalence of right-handers (%)	93	-	-	86	-	-	0.11

Table 2 Workroom air concentrations of Mn measured in personal samples among the Mn-exposed subjects and concentrations of trace elements in whole blood (B) and urine (U) among the Mn-exposed subjects and the referents

Parameter	Exposed (n = 100)					Referents (n = 100)					P
	Arithmetic mean	Geometric mean	Minimum	Maximum		Arithmetic mean	Geometric mean	Minimum	Maximum		
Years exposed to Mn	20.2 <sup>d</sup>	-	2.1	41.0	-	-	-	-	-	-	-
Mn <sub>inhalable</sub> (µg/m <sup>3</sup> )	753	301	9	11,457	-	-	-	-	-	-	-
Mn <sub>respirable</sub> (µg/m <sup>3</sup> ) <sup>a</sup>	64	36	3	356	-	-	-	-	-	-	-
Mn <sub>inhalable (soluble)</sub> (µg/m <sup>3</sup> ) <sup>b</sup>	570	197	9	9,001	-	-	-	-	-	-	-
Mn <sub>respirable (soluble)</sub> (µg/m <sup>3</sup> ) <sup>c</sup>	49	25	2	320	-	-	-	-	-	-	-
B-Mn (nmol/l)	189 <sup>e</sup>	181	84	426	166	160	72	374	0.002	-	0.002
U-Mn (nmol/mmol creatinine)	3.9	0.9	0.1	126.3	0.9	0.4	0.1	13.1	0.58	0.001	<0.001
B-Pb (µmol/l)	0.20	0.16	0.01	1.27	0.15	0.12	0.01	0.58	0.001	0.001	0.001
B-Hg (nmol/l)	20	18	3	67	19	16	3	56	0.39	0.39	0.39
U-Hg (nmol/mmol creatinine)	1.3	1.0	0.1	4.6	1.4	1.0	0.1	10.7	0.42	0.42	0.42

<sup>a</sup>n = 59

<sup>b</sup>n = 97

<sup>c</sup>n = 53

<sup>d</sup>SD = 8.4

<sup>e</sup>1 µg/l = 18.1 nmol/l

performance on the Static Steadiness test adjusted for age and cigarettes/week among the referents and the exposed subjects stratified into three equally large groups according to duration of exposure.

Table 7 shows differences between Mn-exposed and referents when the exposed subjects were stratified into "low", "medium" or "high" B-Mn levels. The exposed subjects with highest B-Mn concentrations showed more tremor than their age-matched referents on the Static Steadiness test.

Mn-exposed current smokers had more tremor on the Static Steadiness test than the remaining subjects. No statistical interaction between smoking and Mn-exposure was found, but statistically significant main effects of smoking and exposure were found (Table 8).

## Discussion

This study examined nervous system functions in long-term exposed workers in Mn-alloy-producing plants. We previously reported increased serum prolactin concentrations in the same population [16]. Exposed workers and referents were employed in plants with similar working conditions and were comparable in terms of important background characteristics. The high participation among the invited subjects could indicate that selection bias is of little importance.

## Exposure

The GM, individual, current exposure to inhalable Mn was 301 µg/m<sup>3</sup>, of which 10.6% (95% CI 8.9–12.5) was found in the respirable aerosol fraction [15]. We found, by parallel sampling in the Mn-alloy-producing plants under study, that the traditional "total dust" closed-face 25-mm or 37-mm plastic samplers (Millipore) seemed to underestimate exposure to inhalable aerosol by a factor of approximately 2, compared to the IOM sampler used in this study [25]. This should be considered when exposure in our study is compared with exposure to total Mn in other studies.

Other neuropsychological studies of Mn-alloy-production workers have reported 270 and 239 µg Mn/m<sup>3</sup> in the furnace area and 124 and 55 µg Mn/m<sup>3</sup> in the maintenance area as GM of "total" Mn [35, 37], approximately 50% being respirable. However, these exposure levels are difficult for one to compare with our results, due to different sampling strategies. GM concentrations of 940 µg/m<sup>3</sup> and 50 µg/m<sup>3</sup> of "total" Mn, measured in personal air samples, were reported in a Mn oxides and salts producing plant and a Mn-ore-milling plant, respectively [47, 7]. The GM levels of respirable Mn in personal samples collected during production of dry alkaline batteries and electrolytic Mn metal were 215 µg/m<sup>3</sup> [48] and 36 µg/m<sup>3</sup> [19], respectively. No sampling strategy details were given in a study of steel workers, which reported mean air concentration of

**Table 3** Neuropsychiatric symptoms: number of symptoms on the Q16 and symptom subscales on the Helsinki symptom questionnaire among the exposed subjects and the referents

Questionnaire	Exposed (n = 100)		Referents (n = 100)		P
	Mean	Minimum-maximum	Mean	Minimum-maximum	
Q16	2.6	0-10	2.4	0-11	0.61
Helsinki symptom questionnaire					
Sleep disturbances	1.5	1-3.3	1.6	1-3.3	0.24
Fatigue	1.8	1-3.6	1.8	1.2-3.2	0.90
Memory and concentration difficulties	1.7	1-3.5	1.7	1-3.5	1.00
Emotional lability	1.5	1-3.3	1.5	1-3.3	0.65
Somatic complaints	1.5	1-3.0	1.5	1-2.4	0.80
Sensory and motor symptoms	1.3	1-2.6	1.3	1-2.8	0.42

**Table 4** Results of tests for cognitive functions among the Mn-exposed subjects and the referents

Test	Exposed (n = 100)			Referents (n = 100)			P
	Mean	SD	Minimum-maximum	Mean	SD	Minimum-maximum	
Verbal test, general intellectual level							
WAIS information (number)	18.8	4.0	6-26	19.9	3.8	9-28	0.06
Cognitive speed, visual scanning							
WAIS digit symbol (number)	45.2	9.3	24-65	46.3	11.2	16-68	0.44
Trail-making test, A (s)	32.6	11.5	14-67	30.7	12.3	15-93	0.25
Trail-making test, B (s)	85.7	36.6	36-225	87.6	45.0	33-248	0.75
Stroop, colour dots <sup>a</sup> (s)	30.4	7.1	20-65	29.2	7.7	19-75	0.30
Stroop, reading task (s)	18.7	3.4	13-30	17.8	3.6	13-30	0.07
Stroop, interference colour/word <sup>a</sup> (s)	58.2	15.6	37-105	57.5	24.3	36-224	0.81
Attention, short-term memory							
WAIS digit span—forwards (number)	6.1	1.1	4-9	6.2	1.1	4-8	0.57
WAIS digit span—backwards (number)	5.0	1.4	2-8	5.1	1.4	3-8	0.66
Benton, correct (number)	6.7	1.6	2-10	6.7	1.6	2-10	0.73
Benton, error (number)	5.0	2.9	0-15	4.6	2.5	0-11	0.25

<sup>a</sup>Log transformed (log 10) values used for statistical comparisons

**Table 5** Results of the tremor tests for the Mn-exposed subjects and the referents

Parameter	Exposed (n = 100)			Referents (n = 100)			P
	Mean	SD	Minimum-maximum	Mean	SD	Minimum-maximum	
Klve-Matthews' static steadiness test							
Number of contacts <sup>a</sup>	94 <sup>b</sup>	93	5-697	59 <sup>c</sup>	51	0-218	0.001
Duration of contacts (s)	5.1	4.4	0.2-19.3	3.5	3.4	0-16.8	0.003
DPD TREMOR:							
Dominant hand							
Tremor intensity (m/s <sup>2</sup> )	0.13	0.06	0.05-0.40	0.14	0.05	0.07-0.28	0.50
Frequency (Hz)	7.4	1.1	4.8-10.3	7.4	1.1	4.9-10.3	0.72
Frequency dispersion (Hz)	3.1	0.8	1.0-5.2	2.6	0.9	1.0-4.5	<0.001
Non-dominant hand							
Tremor intensity (m/s <sup>2</sup> )	0.13	0.05	0.07-0.40	0.13	0.04	0.05-0.25	0.49
Frequency (Hz)	7.7	1.3	3.8-11.6	7.8	1.2	5.1-11.2	0.58
Frequency dispersion (Hz)	3.3	0.8	1.6-5.1	3.1	0.8	0.6-4.9	0.08

<sup>a</sup>Arithmetic mean; Log transformed (log 10) values used for statistical comparisons

<sup>b</sup>Geometric mean 65

<sup>c</sup>Geometric mean 40

0.25 mg Mn/m<sup>3</sup> [29]. Between 20% and 80% was suggested to be in the respirable aerosol fraction [53].

Previous exposure in the plants was, unfortunately, not well documented, but there are indications, at least in the largest of the three plants, that exposure was two- to three-times higher previously [26].

#### Motor tests

##### Tremor

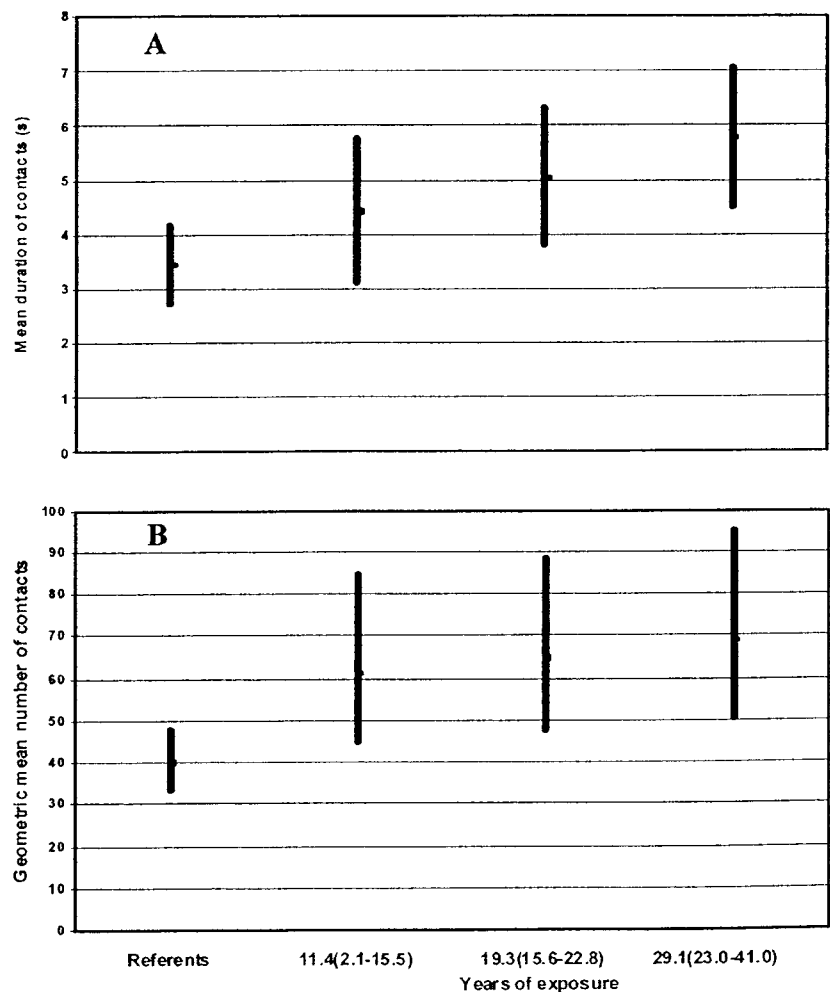
Tremor can be defined as "a rhythmical, involuntary, oscillatory movement of any body part" [18] and is

**Table 6** Tests for manual dexterity, coordination, grip strength, motor speed and reaction time among the Mn-exposed subjects and the referents

Test	Exposed ( <i>n</i> = 100)			Referents ( <i>n</i> = 100)			<i>P</i>
	Mean	SD	Minimum–maximum	Mean	SD	Minimum–maximum	
Pegboard, dominant hand (s)	69.7	11.2	52–110	69.0	13.9	47–132	0.67
Pegboard, non-dominant hand (s)	76.4	12.3	56–115	73.8	12.8	51–120	0.15
Luria, thumb/finger-sequential touch—dominant hand (number)	7.8	1.7	5–15	8.0	1.8	5–18	0.38
Luria, thumb/finger-sequential touch—non-dominant hand (number)	7.7	1.6	4–13	7.9	1.8	4–17	0.33
CATSYS, DPD; Coordination test							
Maximum frequency (Hz)	4.7	1.1	2.6–7.5	5.0	1.2	2.8–7.6	0.047
Maximum frequency; performance index	0.86	0.54	0.11–1.92	1.02	0.58	0.13–1.93	0.04
Dynamometer—dominant hand (kg)	55.4	8.2	35–78	53.5	7.9	32–74	0.11
Dynamometer—non-dominant hand (kg)	52.8	7.7	29–69	51.3	7.5	32–70	0.16
Finger tapping (number)	50.1	5.5	32–64	50.7	5.4	31–62	0.47
Foot tapping (number)	45.6	6.9	31–66	46.1	7.0	25–66	0.64
NES2 HECT	1.84	0.32	1.22–2.85	1.82	0.38	1.04–2.75	0.74
NES2; SRT <sup>a</sup> (ms)	236	28	189–339	233	33	179–435	0.53
NES2; SD of SRT (ms)	37	12	18–72	35	13	13–83	0.31

<sup>a</sup>Log transformed (log 10) values used for the statistical comparisons

**Fig. 1** The Static Steadiness test performance (*panel A* duration of contacts; *panel B* number of contacts) adjusted for age and cigarettes/week among referents and exposed subjects, stratified into three equally large groups according to duration of exposure



**Table 7** Tremor measures for dominant hand, adjusted for tobacco consumption and age, according to levels of B-Mn in the exposed subjects: "low" (<157 nmol/l), "medium" (157–203 nmol/l),

"high" (>203 nmol/l). The comparisons are based on pair-wise matching of Mn-exposed subjects (*E*) with the age-matched referents (*R*) (*n* = 200). *AM* arithmetic mean, *GM* geometric mean

Parameter	"Low" B-Mn			"Medium" B-Mn			"High" B-Mn		
	E ( <i>n</i> = 34) Mean	R ( <i>n</i> = 34) Mean	<i>P</i>	E ( <i>n</i> = 32) Mean	R ( <i>n</i> = 32) Mean	<i>P</i>	E ( <i>n</i> = 34) Mean	R ( <i>n</i> = 34) Mean	<i>P</i>
Age	44.3	44.3	1.0	44.1	44.1	0.97	44.1	44.2	1.00
Tobacco consumption (g/week)	37	40	0.83	52	39	0.43	35	43	0.54
Duration of exposure (years)	18.0	—	—	21.2	—	—	20.0	—	—
B-Mn (nmol/l)	135 <sup>a</sup>	153	0.02	180	166	0.09	253	178	<0.001
Kl $\ddot{o}$ ve Matthews static steadiness test									
Number AM	78	65	—	75	57	—	127	56	—
Number GM	58	40	0.12	55	40	0.13	87	42	<0.001
Duration (s)	4.6	4.1	0.54	4.4	3.0	0.08	6.4	3.2	0.003
"TREMOR"									
Frequency dispersion	2.9	2.7	0.24	3.2	2.6	0.04	3.2	2.5	<0.001

<sup>a</sup>1  $\mu$ g/l = 18.1 nmol/l

**Table 8** Results from tremor measurements (adjusted for age) among smokers and among non-smokers according to exposure to Mn

Parameter	Smokers ( <i>n</i> = 95)		Non-smokers ( <i>n</i> = 105)		<i>P</i> <sup>a,b,c</sup>
	Exposed ( <i>n</i> = 50) Mean (95% CI)	Referents ( <i>n</i> = 45) Mean (95% CI)	Exposed ( <i>n</i> = 50) Mean (95% CI)	Referents ( <i>n</i> = 55) Mean (95% CI)	
Age	44.0(41.4–46.7)	43.9(41.2–46.6)	44.3(41.8–46.9)	44.4(42.0–46.9)	—
Static steadiness test					
Dominant hand					
Duration of contacts	6.1(5.1–7.1)	3.8(2.7–4.9)	4.1(3.1–5.1)	3.2(2.2–4.2)	0.002, 0.01, 0.19
Number of contacts <sup>d</sup>	85(67–109)	43(33–56)	50(39–64)	38(30–48)	<0.001, 0.008, 0.10
TREMOR 7.0					
Dominant hand					
Tremor intensity	0.15(0.14–0.17)	0.15(0.14–0.17)	0.11 (0.10–0.13)	0.13 (0.12–0.14)	0.39, <0.001, 0.27
Frequency dispersion (Hz)	2.9(2.6–3.1)	2.4(2.2–2.7)	3.3 (3.1–3.6)	2.8 (2.5–3.0)	<0.001, 0.001, 0.62
Non-dominant hand					
Tremor intensity	0.15(0.14–0.16)	0.12(0.10–0.13)	0.12(0.10–0.13)	0.12(0.11–0.14)	0.52, 0.003, 0.09
Frequency dispersion (Hz)	3.1(2.9–3.3)	3.2(2.9–3.4)	3.5(3.3–3.7)	3.0(2.8–3.3)	0.09, 0.22, 0.03

<sup>a</sup>*P* for the separate effect of Mn-exposure

<sup>b</sup>*P* for the separate effect of smoking

<sup>c</sup>*P* for the interaction term smoking  $\times$  Mn-exposure

<sup>d</sup>Geometric mean

associated with rhythmical, synchronous, neuronal discharges in various basal ganglia [42]. The increased tremor measured by the Static Steadiness test is in accordance with other studies where hole-tremometers were used [27, 41, 47, 48]. Reported associations between increased tremor and B-Mn [8, 47], lifetime integrated exposure to total Mn dust [48], or years of Mn exposure [8], also conform to our results. Higher central frequency and smaller frequency dispersion of tremor reported among Mn-alloy plant workers [37], or decreasing frequency dispersion associated with increasing B-Mn concentrations [5], is not supported by our results. It cannot be excluded that some of the subjects might have an essential tremor, although the occurrence of essential tremor, whether familial or not, should be evenly distributed in the two groups.

We used the Static Steadiness test and the "TREMOR" test to measure *postural tremor*, defined as tremor present while the subject maintains position against

"gravity" [18]. Postural tremor may appear or become exacerbated in specific, usually visually guided, tasks. This is a characteristic of the Static Steadiness test, but does not apply to "TREMOR". The tremor measured by the Static Steadiness test may be called "position-specific postural tremor" [18]. One explanation of the difference in findings between the two tremor tests may be the difference in test instruments.

The Mn-exposed subjects showed a tremor with a larger frequency dispersion, indicating that the tremor included a wider variety of frequencies, more like a physiological tremor. This tremor may be more adequately recorded by the Static Steadiness test equipment, which records displacement, in contrast to the "TREMOR" test, which records acceleration. A third explanation may be the different test times. The test time for the Static Steadiness test was 135 s. The "default" test time of 8.2 s for the "TREMOR" may have been too short to elicit tremor and could, therefore, have made

the test less sensitive. In recent studies using "TREMOR", the test time was increased [12] or the subjects were required to repeat the test [33] in order to increase reproducibility and accuracy.

#### *Smoking and tremor*

Self-reported smoking habits had an impact on many tremor measures. A misclassification of self-reported smoking habits is imaginable. A non-differential misclassification of exposure (smoking habits) would have biased the results towards the null [32]. If misclassification of smoking habits in our study is assumed, smokers are probably more likely to report that they are non-smokers rather than non-smokers report they are smokers. This would also bias the impact of smoking towards the null effect. In contrast to the larger frequency dispersion associated with manganese exposure, smoking was associated with a smaller frequency dispersion, indicating a more narrow variation of frequencies. The effect of smoking habits on the tremor measurements is small and probably of no clinical relevance.

The association between tobacco smoking and tremor has received little attention in occupational studies. Recent studies reported increased tremor measured by the Static Steadiness test or tremor with a lower central frequency in smokers [14, 5]. More self-reported motor symptoms, including tremor, was reported among Mn-exposed subjects smoking cigarettes at work [31]. Animal studies have assessed the potential effects on tremor of a combined administration of nicotine and other neurotoxicants. Rats exposed to large quantities of ethanol and then withdrawn from ethanol exposure had more tremor after injection of high doses of nicotine than rats exposed to nicotine only [22]. Physostigmine potentiated nicotine-induced tail tremor in rats [21].

#### *Other tests for motor function and reaction time*

No group differences were found in tests of motor speed, grip strength or reaction time. On the CATSYS supination/pronation test, there was a statistically significant difference between exposed subjects and referents, but no association with exposure measures was found. We also observed that the drum did not always lie firm, which may have made the test results less valid. Recently, Despres et al. [12] reported building a holder in order to increase the stability of the drum. No difference between the groups for the HECT was found. Roels et al. [47, 48] reported an effect on hand-eye coordination. Their HECT (orthokinesometer) is different from the HECT used in our study. The orthokinesometer has been described as testing a combination of hand-eye-coordination and kinetic tremor [46], while the HECT includes elements of attention and speed.

#### *Cognitive tests*

None of the cognitive tests discriminated significantly between the groups. The slightly weaker results of the exposed subjects on the information subtest are compatible with their 0.5-years' shorter education. The information test score was our a priori selected covariate for adjusting for general intellectual ability [14]. This question has been addressed differently in earlier Mn studies. Some studies report cognitive effects from Mn exposure without adjustment for "pre-exposure" general intellectual abilities [6, 47]. Some studies with considerable differences in cognitive level or amount of education adjust statistically for these differences and still find substantial effects of exposure [35, 37]. Some studies report few [41] or no effects on cognitive tests [8, 29, 48, 50].

#### *Neuropsychiatric symptoms*

The results do not indicate an increase in neuropsychiatric symptoms. This is in accordance with some studies [19, 48]. Most studies report a moderate [6, 29, 37, 47, 50] or a substantial increase in symptoms [41]. Shift work was significantly related to number of symptoms on the Q16 and to the "memory and concentration difficulties" scale on the Helsinki questionnaire. With one exception [37], shift work does not seem to have been addressed as a potential confounder for symptom reporting in published Mn toxicity studies.

#### *Conclusion*

The Mn-exposed subjects showed increased postural tremor when executing a visually guided task, compared with the referents. The tremor was related to duration of exposure, but too few historical air measurements were available to allow the calculation of a cumulative exposure index. Smoking habits (self-reported) also had an impact on the tremor parameters. The results indicate that, among the applied neuropsychological methods, tests for tremor appear to be the most sensitive methods for the detection of early effects on the nervous system from Mn exposure. No effect of exposure was found in tests for cognitive functions, reaction time or in symptom reporting.

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